PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 98,375-C)

In the Application of:	)	
	)	
Mehta et al.	)	
	)	**Group Art Unit: 1648**
Serial No.: 09/701,979	)	
	)	**Examiner: Lucas, Zachariah**
Filed: February 12, 2001	)	
	)	
For: METHOD FOR STAINING BIOLOGICAL SPECIMENS BY COMBINING UNSTABLE REAGENTS ON A MICROSCOPIC SLIDE	)	

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Appeal Brief is submitted in accordance with the requirements of 37 CFR 41.37. The fee required by 37 CFR 41.20(b)(2) is submitted herewith.

I. REAL PARTY IN INTEREST

The real party in interest of this pending application is Ventana Medical Systems, Inc. which is the owner by Assignment of the above-identified U.S. patent application.

II. RELATED APPEALS AND INTERFERENCES

There are no Appeals or Interferences related to the above-identified U.S. Patent Application.

III. STATUS OF THE CLAIMS

Claims 1-2 and 4-13 are pending in this application. Claim 3 stands cancelled. A copy of currently pending claims 1-2 and 4-13, involved in this appeal, are attached hereto as Appendix A.

IV. STATUS OF AMENDMENTS

The examiner rejected all pending application claims 1-2 and 4-13 in a Final Rejection issued on July 27, 2004. There are no amendments outstanding. No claims have been allowed by the examiner.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The present invention is directed generally to methods for staining biological samples using unstable staining solutions. More particularly, the present invention is directed to methods for applying individual stable components of unstable histochemical solutions to a biological sample of interest and thereafter combining the stable solutions directly on the biological material to form an unstable staining solution. (Page 3, lines 8-16).

Staining solutions are commonly used to identify certain substances and/or structures within or outside biological materials such as cells. Many biological stains are unstable, toxic and generally are difficult to work with. Prior to the current invention, automated and manual histochemical staining methods and apparatus required the premixing of two or more solutions to form a stain prior to applying the stain to sample tissues. In many cases, the mixing of several ingredients to prepare a staining solution forms a staining solution that is inherently unstable. This instability may manifest itself by the appearance of precipitates or films in the staining solution. For example, many silver staining solutions are photolabile. Such solutions degrade rapidly and silver resident can observed on the top of the solution within hours of mixing. The formation of films and precipitates in unstable staining solutions may negatively affect the staining of the tissues and therefore decrease the accuracy of histochemical testing. In order to avoid these problems, unstable staining solutions are prepared daily if not more frequently. Frequent preparation of

unstable staining solutions is time consuming and it results in the loss of expensive staining solutions if the staining solution is not used before becoming unstable. (Page 2, lines 4-17).

The presently claimed invention is directed to methods whereby two or more stable ingredients of an unstable staining solution are sequentially applied to a biological sample where they are allowed to form an unstable staining solution. (*E.g.*, page 4, lines 10-12). The formation of an unstable staining solution *in-situ* may be accelerated by mixing the first and second stable solutions after they are applied to the biological material. (*E.g.*, page 4, lines 13-17). Mixing can be accomplished by applying, for example, a gas stream that is directed at the admixture of the first and second stable solutions once they are applied to the biological material. (*E.g.*, page 10, line 22 to page 11, line 9).

The claimed staining methods are useful for applying a variety of unstable staining solutions including, but not limited to fungi staining solutions, silver staining solutions, iron staining solutions, iron hematoxylin solutions, trichrome staining solutions, mucin stains, mucicarmine staining solutions, Verhoff's staining solution, amyloid staining solutions, and Steiner staining solution to biological materials. (*E.g.*, page 3, lines 17-24). The methods of this invention are especially useful for automating the staining of a biological material with unstable staining solutions such as silver staining solutions (*See, e.g.*, claims 6-7; page 6, line 17 to page 7, line 17), trichrome or mucicarmine staining solutions (*See, e.g.* claim 8; page 7, lines 18-21) and iron staining solutions (*See, e.g.*, claim 9; page 8, lines 7-9).

The methods of the present invention are useful for staining a variety of biological materials including those selected from the group consisting of tissue sections, tissue culture cells, smears of blood, spitum, body fluids, excretions, secretions, micro-organisms as well as cell components selected from the group consisting of cell organelles or ganelles, chromosomes, nucleonic acids, carbohydrates, liquids and proteins. (*E.*g., page 11, lines 10-14).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The examiner rejected claims 1-2 and 4-13 for allegedly being unpatentable under 35 U.S.C. § 103(a) for obviousness over McCormick (USP No. 3,431,866) in view of Copeland (USP No. 5,650,327) and in view of McManus et al. (Staining Methods, Histologic and Histochemical, Paul B. Hober, Inc. New York, 1960) and Stokes et al. (USP 5,318,795).

It is the examiner's position that McCormick teaches an automated method for staining materials on the slide including steps of providing a plurality of staining solutions, providing the slide having biological materials, and providing an automated delivery system to deliver a predetermined amount of staining solution to the slide and sequentially applying staining solutions to the slide. (November 18, 2003 Official Action at page 3). The examiner acknowledges that McCormick does not teach mixing liquids on the slides using gas streams. *Id.*

The examiner relies on Copeland for teaching automated methods for staining tissue sections mounted on slides that use gas streams to mix solutions on the surface of the slide. The examiner admits that Copeland does not specifically teach that the staining reagent being mixed is an unstable staining resulting from the mixing of two or more stable reagents. It is the examiner's position that unstable stains made from stable ingredients are known in the art as evidenced by McManus and by the applicants' description of conventional unstable stains in the art. *Id.*

Finally, the examiner relies upon Stokes to support his allegation that the claimed invention is obvious to those of ordinary skill in the art. The examiner characterizes Stokes as being concerned with automated methods for staining biological samples. The examiner refers to the explicit statement in the reference that "where the claims are directed to a step in the staining process, it is understood that the step may comprise a single step or a combination if the reagents are applied or sequential steps where more than one reagent of combination of reagent is applied" for teaching that multiple reagents may be applied as part of a single reagent application step. (Final Rejection at page 3).

VII. ARGUMENT

A. The Prior Art

1. The McCormick Reference (USP 3,431,866)

The McCormick reference discloses apparatuses that are useful for applying solutions, including staining solutions, to materials located on microscope slides. The McCormick reference is generally directed to describing features of an apparatus. The reference does, however, refer by Example to a staining procedure that is performed on the apparatus. (Col. 6, lines 57-61). This excerpt of McCormick states:

> It is to be understood, of course that any number of such stations may provide and its numbers shown is only illustrative of a particular staining

procedure, namely, the Wright procedure for staining blood smears and in carrying out the Wright procedure in the approve apparatus, a staining liquid is applied at the first station, a buffer liquid is applied at the second station and a washing liquid is applied at the third station.

- The McCormick reference is notable because it does not disclose applying ingredients of a useful reagent independently to a biological sample.
- It does not disclose applying more than one reagent or biological sample before washing the reagent from the sample prior to application of a subsequent reagent to the sample.

2. The Copeland et al. Reference (USP 5,650,327)

The Copeland et al. patent discloses an automated immunostaining apparatus. The apparatus includes a gas stream that is directed at liquids that are in contact with biological samples located on sides in order to stir the liquids. (Col. 3, lines 53-55). The Copeland et al reference is generally directed to describing the components of the automated apparatus. One apparatus function - its use in an immunohistological method - is described at columns 19-20 of the patent. The immunohistological method is described as having the following nine steps:

Step 1 - Slide separation.
Step 2 - Inserting a batch of slides in the apparatus.
Step 3 - Closing the apparatus and beginning processing.
Step 4 - Rinsing a slide in the first rinse station, beginning the rinsing up to seven times and applying an evaporation inhibiting liquid to the slide.
Step 5 - Moving the slide into a reagent application zone and applying a metered volume of a reagent to the slide.
Step 6 - Passing the slide to the gas vortex mixing station.
Step 7 - Moving the slide to the initial rinse station at step 4.
Step 8 - Repeating steps 4-7 to complete a four phase process in which four different reagents are applied to the slide, allowed to remain in contact with the material on the slide for a defined period of time and thereafter rinsed from the slide in the rinse station before a next reagent is applied to the slide.
Step 9 - Removing the slide from the apparatus to determine the extent of binding.

Copeland et al does not disclose:

- Applying two ingredients of a reagent on the slide to form a single reagent that reacts with the biological material.

- Applying two or more reagents to a slide before rinsing the slide in preparation for the next reagent application step.
- Sequentially applying ingredients of unstable staining solutions to a biological sample.

3. The McManus Article

The McManus article lists the recipes for several unstable staining solutions. The McManus article further discloses that unstable staining solutions are manufactured from stable ingredients.

4. The Stokes et al. Reference (USP 5,318,795)

The Stokes et al. reference is directed generally to methods for automated treatment of biological samples including staining biological samples. More particularly, the Stokes reference discloses applying reagents, such as stains, to biological samples by spray methods. Examples of spray staining procedures are disclosed in Figures 1 and 2 and the accompanying specification description.

The excerpt of the Stokes reference relied upon by the examiner states:

> Where the claims are directed to a step in the staining process, it is understood that the step may comprise a single step where a combination of reagents are applied with sequential steps for more than one reagent or combination of reagents is applied. (Col. 10, lines 34-38).

It is important however to understand this statement in context of the Stokes reference in its entirety. The excerpt does not represent the knowledge of one or ordinary skill in the art at the time of the invention. Importantly, Stokes does not disclose the following features of the claimed invention:

- Applying ingredients of a reagent composition independently to a biological sample to form a reactive reagent on the biological sample.
- Stokes does not disclose applying ingredients of unstable staining solutions independently to a biological sample.

B. Errors In The Final Obviousness Rejection

Claims 1-2 and 4-13 stand finally rejected under 35 U.S.C. 103(a) as being unpatentable over McCormick in view of Copeland and in view of McManus et al. (Staining Methods, Histologic and Histochemical, Paul B. Hober, Inc. New York, 1960) and Stokes et al. (USP

5,318,795).

Pending application claims 1-2 and 4-13 are patentable over the prior art of record because the examiner has not made out a *prima facie* case of obviousness of the claims cover the cited prior art. During patent examination the PTO bears the initial burden of presenting a *prima facie* case of unpatentability. *In re Oetiker*, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); *In re Piasecki*, 223 USPQ 785, 788 (Fed. Cir. 1984). If the PTO fails to meet this burden, then the Appellant is entitled to the patent. *See In re Glaug*, 62 USPQ2d 1151 (Fed. Cir. 2002). A *prima facie* case of obviousness has not been established with respect to the pending claims because: (1) none of the prior art references disclose or suggest the claimed methods; (2) the examiner's obviousness rejection relies upon an improper hindsight analysis of the prior art.

1. The prior art does not disclose or suggest that ingredients of an unstable staining solution can be individually applied to a biological sample

The examiner admits in his First Official Action and in his Final Rejection that the prior art of record does not disclose separately applying two stable ingredients of an unstable staining solution to a biological sample and thereafter forming an unstable staining solution in contact with the biological sample. Yet the examiner concludes that the claims are obvious nonetheless because (1) one of ordinary skill in the art would have been led by their own knowledge to combine the references; and (2) after combining the references, the claimed invention would have been obvious to one of ordinary skill in the art.

A *prima facie* case of obviousness cannot be constructed without showing that all of the features of the claimed invention are found in the prior art. *See e.g., In re Rouffet,* 199 Fed.3d 1350, 1359 (Fed. Cir. 1998). In fact there is no teaching in any cited prior art references that could hypothetically be combined to lead one of ordinary skill in the art to arrive at Appellant's invention. And, even if there were such teachings, there would be no motivation for one of ordinary skill in the art to combine them to arrive at the claimed invention.

The applicant's position in favor of claim patentability is quite simple – the prior art of record only discloses applying premixed staining solutions to biological samples. The prior art staining solutions do not require the addition of further ingredients or mixing in order to be useful. The prior art does not disclose or suggest the applicants invention – that stable ingredients that

form a staining solution can be individually applied to a biological sample and then mixed while in contact with the biological sample to form a useful staining solution.

The examiner has not established a *prima facie* case of obviousness because none of the cited prior art references disclose or suggest adding individual ingredients of any solutions – staining solutions or otherwise – to a biological sample during any type of biological treatment procedures including staining procedures. The Examiner has cited a single sentence passage from Stokes et al. for suggesting this teaching. However, the Examiner's reliance on the Stokes et al. passage is misplaced because he interprets the single sentence of Stokes et al. in a manner that is inconsistent with the sentence and with the reference as a whole.

The Examiner cited column 10, lines 34-38 of Stokes et al. for disclosing the concept that "multiple reagents may be applied as part of the single staining step". (See page 5 of the November 18, 2003 Official Action). The cited excerpt states:

> "Where the claims are directed to a step in the staining process, it is understood that the step may comprise a single step where a combination of reagents are applied or sequential steps where more than one reagent or combinations of reagents is applied."

A complete reading of Stokes et al. shows that the examiner has misinterpreted this excerpt of Stokes et al. Moreover, the examiner has, without support, alleged that his interpretation of the Stokes excerpt is demonstrative of the knowledge of one of ordinary skill in the art at the time of the applicant's invention.

In order to understand the meaning of this sentence, one must consider the Stokes et al. reference as a whole. Stokes et al. is directed to a method for replacing a procedure whereby biological samples on slides undergoing staining are sequentially dipped into solutions during staining procedures with a process in which reagents are sequentially sprayed onto slides. Figures 1 and 2 and Examples 1 and II and the specification of Stokes et al. disclose methods for applying a sequence of solutions, including pre-mixed staining solutions, to biological samples located on slides. Figure 1of the Stokes et al. reference is reproduced below. Figure 1 identifies the steps of the method for automated staining of cytology specimens. It is clear from Figure I that the staining method includes steps of applying the reagents A – F to the specimen in order to achieve the required staining results.

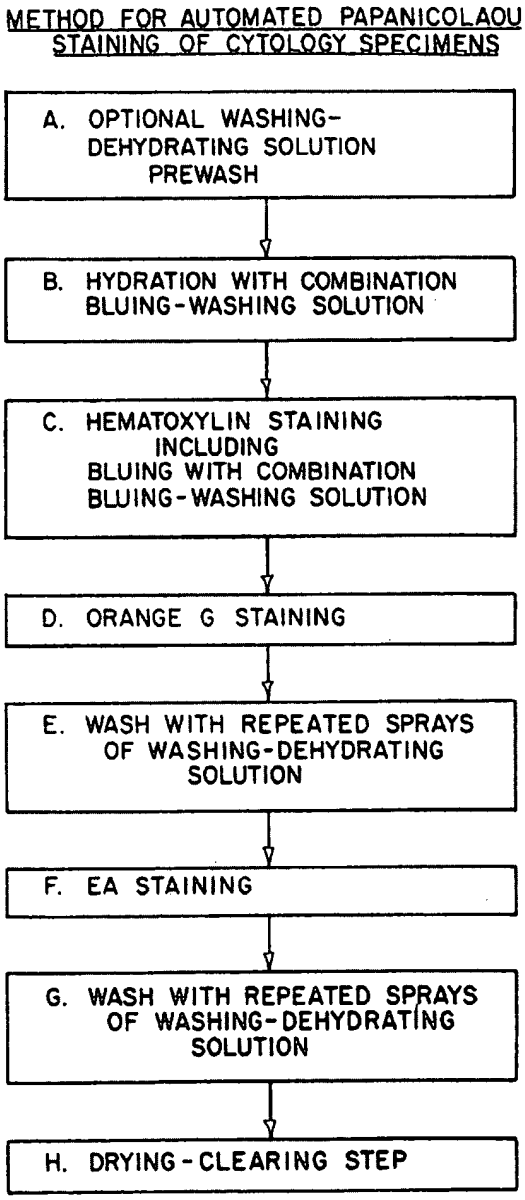


FIG. 1

Nowhere does Stokes et al. disclose or suggest that individual staining solution ingredients can be independently applied to the biological samples by spraying. Instead, upon reading Stokes et al. in its entirety, it becomes clear that what is taught by the excerpt quoted and relied upon by the examiner is that, for purposes of the claims – the multiple steps of for example, the staining

procedure of Example 1, may together be considered a single "step" for purposes of claiming the invention. The excerpt cited by the examiner cannot in the context of the Stokes et al. patent be construed to mean – as the examiner suggests - that multiple reagents "ingredients" may be applied as part of a single staining step to form a staining solution because there is absolutely no disclosure in Stokes et al. that would support this interpretation of the cited statement. Instead, the cited passage refers to the application of several reagents to a specimen during a staining procedure.

Moreover, the cited passage merely defines how a term of the claims is to be interpreted. In addition to improperly construing this passage, the examiner has taken the position that one of ordinary skill in the art would understand the passage to apply to all other prior art of record at the time of the invention. Once again, that is not the case. The passage has a limited application defining the terms of the Stokes et al. claims. Clearly, one of ordinary skill in the art would not understand the definition to apply to the prior art in general

2. The obviousness rejection is based upon an improper hindsight analysis of the prior art

There is no teaching or suggestion in the prior art cited the Examiner that ingredients of an unstable staining solution my be applied independently and before mixing to a biological sample and thereafter allowed to form an unstable staining solution in contact with the biological material. The examiner takes the position that the term "reagent" as used in the prior art refers equally to ingredients of unstable staining solutions and that one of ordinary skill in the art would understand the term reagent in the prior art includes such ingredients. There is no disclosure or suggestion in the prior art that the term "reagent" as used in the prior art encompasses individual ingredients that together form a useful solution. Instead, the term "reagent" is used throughout the prior art to refer to solutions, that are useful alone – and without the addition of further ingredients – to perform a procedure or a procedure step.

What the Examiner has done is to evaluate the prior art in hindsight. In rejecting all claims for obviousness, the Examiner has clearly first considered the Applicants' invention and then has viewed the prior art references in hindsight – with the Applicant's invention in mind. This is not the correct obviousness analysis. "Measuring a claimed invention against the standard established by section 103 requires the oft-difficult but critical step of casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by

the prior art references and the then-accepted wisdom in the field." *See, e.g., W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 UPSQ 303, 313 (Fed. Cir. 1983). Adherence to this methodology is important where the very ease with which the invention can be understood may prompt one "to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." *Id.*

The prior art does not support the examiner's interpretation that the term "reagent" to include ingredients of a useful solution. Nowhere does the prior art use the term reagent in this context. The examiner's citation of an excerpt from a disclosure on a "periodic acid Schiff reaction" does not alter this conclusion because the Examiner sought out this definition, in hindsight, in order to force the prior art to be interpreted in a manner that is contrary to the teaching of each of the references. When considered for their teachings alone, one of ordinary skill in the art would not understand the references cited by the examiner to teach or suggest the claimed invention. Instead, one of ordinary skill in the art would understand that the cited references, either alone or when combined, disclose that pre-mixed unstable staining solutions of McManus or the pre-mixed staining solutions of Stokes et al. (and not individual staining solution ingredients) can be applied to samples using the methods and apparatuses of Stokes et al., McCormick et al. and Copeland et al. Clearly, the examiner's rejection relies upon an improper hindsight analysis of the prior art. As a result, the obviousness rejection of all application claims should be withdrawn.

CONCLUSION

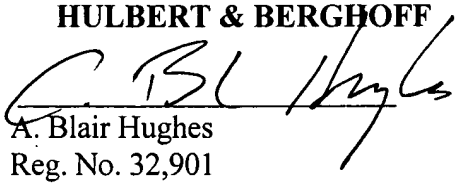
The examiner's obviousness rejection of claims 1-2 and 4-13 cannot be sustained by the Board because the examiner has failed to establish a *prima facie* case of obviousness. Specifically, there is no *prima facie* case of obviousness of claims 1-2 and 4-13 because there is no teaching or suggestion in the prior art of record of applying stable ingredients of unstable solutions directly to a biological sample and thereafter forming an unstable solution in contact with the biological sample. Moreover, there is no *prima facie* case of obviousness because the examiner has rejected the claimed after interpreting the prior art in hindsight with the applicant's invention in mind.

Each of these positions in favor of the patentability provides an independent ground for the Board to overrule the examiner's obviousness rejection of claims 1-2 and 4-13.

Respectfully submitted,

McDONNELL BOEHNEN HULBERT & BERGHOFF

Dated: May 6, 2005

By: 

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APPENDIX A

Listing of Claims:

1. (Previously presented) An automated method for staining biological materials on a slide, comprising:

a) providing at least a first and second stable solution, wherein the at least first and second stable solutions form an unstable staining solution when combined;

b) providing a slide, wherein a biological material to be stained is present on the slide; and

c) sequentially applying the at least first and second stable solutions to the biological material on the slide using an automated delivery system to form an unstable staining solution in contact with the biological material.

2. (Original) The method of claim 1 further comprising mixing the at least first and second stable solutions on the biological material.

3. (Cancelled)

4. (Original) The method of claim 1 wherein said unstable staining solution is selected from the group consisting of fungi staining solutions, silver staining solutions, iron staining solutions, iron hematoxylin solutions, trichrome staining solutions, mucin stains, mucicarmine staining solutions, Verhoff's staining solution, amyloid staining solutions, and Steiner staining solution.

5. (Original) The method of claim 2 wherein the mixing is accomplished by applying a gas stream to the at least first and second stable solutions on the biological material.

6. (Original) An automated method for silver staining biological materials on a slide, comprising:

a) providing a first stable solution of from about 0.2% to about 1.0% silver nitrate;

b) providing a solution of from about 2.0% to about 4.0% methenamine;

c) providing a solution of from about 0.2% to about 0.6% borax;

d) providing a slide, wherein a biological material to be stained is present on the horizontal slide;

e) providing an automated delivery system to deliver a predetermined quantity of the silver nitrate, methenamine, and borax solutions to the biological material on the slide;

f) sequentially applying the silver nitrate, methenamine, and borax solutions to the biological material on the slide using the automated delivery system; and

g) mixing the silver nitrate, methenamine, and borax solutions to form an unstable staining solution associated with the biological material.

7. (Original) An automated method for silver staining biological materials on a slide, comprising:

a) providing a solution of from about 0.2% to about 1.0% silver nitrate;

b) providing a solution of from about 0.3% to about 1.0% ammonium hydroxide

c) providing a solution of from about 0.7% to about 1.5% sodium hydroxide

d) providing a slide, wherein a biological material to be stained is present on the slide;

e) providing an automated delivery system to deliver a predetermined quantity of the silver nitrate, ammonium hydroxide, and sodium hydroxide solutions to the biological material on the slide;

f) sequentially applying the silver nitrate, ammonium hydroxide, and sodium hydroxide solutions to the biological material on the slide using the automated delivery system; and

g) mixing the silver nitrate, ammonium hydroxide, and sodium hydroxide solutions to stain the biological material.

8. (Original) An automated method for trichrome or mucicarmine staining of biological materials on a slide, comprising:

a) providing a solution of from about 0.7% to about 1.5% hematoxylin;

b) providing a solution of from about 0.5% to about 1.5% aqueous ferric chloride

c) providing a slide, wherein a biological material to be stained is present on the slide;

d) providing an automated delivery system to deliver a predetermined quantity of the hematoxylin and aqueous ferric chloride solutions to the biological material on the slide;

e) sequentially applying the hematoxylin and aqueous ferric chloride solutions to the biological material on the slide using the automated delivery system; and

f) mixing the hematoxylin and aqueous ferric chloride solutions to stain the biological material.

9. (Original) An automated method for iron staining of biological materials on a slide, comprising:

a) providing a solution of from about 8% to about 12% potassium ferrocyanate;

b) providing a solution of from about 15% to about 30% hydrochloric acid

c) providing a slide, wherein a biological material to be stained is present on the slide;

d) providing an automated delivery system to deliver a predetermined quantity of the potassium ferrocyanate and hydrochloric acid solutions to the biological material on the slide;

e) sequentially applying the hematoxylin and aqueous ferric chloride solutions to the biological material on the slide using the automated delivery system; and

f) mixing the potassium ferrocyanate and hydrochloric acid solutions to stain the biological material.

10. (Previously presented) The method of claim 1 wherein said biological material is selected from the group consisting of tissue sections, tissue culture cells, smears of blood, sputum, body fluids, excretions, secretions, and micro-organisms.

11. (Previously presented) The method of claim 10 wherein the biological material is a cell component that is selected from the group consisting of cell organelles, chromosomes, nucleic acids, carbohydrates, lipids, and proteins.

12. (Previously presented) The method of claim 10 wherein the biological material is a micro-organism that is selected from the group consisting of parasites, viruses, bacteria, and fungi.

13. (Previously presented) An automated method for staining biological materials on a slide, comprising:

a) providing at least a first and second stable solution, wherein the at least first and second stable solutions form an unstable staining solution when combined;

b) providing a slide, wherein a biological material to be stained is present on the slide; and

c) sequentially applying the at least first and second stable solutions to the biological material on the slide using an automated delivery system wherein the sequential application comprises the steps of (i) applying the at least first stable solution to the biological sample and (ii) applying the at least second stable solution to the biological sample without rinsing the biological sample in between application steps (i) and (ii).